M2-IM were able to induce fibroblast activation *in vitro* mediated by an enhanced TGF-β1 expression suggesting a profibrotic role of M2-IM. Specific depletion of hybrid AM using intranasal administration of clodrosome increased radiation-induced fibrosis score and enhanced M2-IM infiltration suggesting a protective role of hybrid AM.

Conclusion: These present study shows a dual and opposite contribution of alevolar *versus* intertitial macrophages in radiation-induced fibrosis and identify M2-IM as a potential therapeutic target to treat radiation-induced fibrosis.

EP-2045

In vivo monitoring of skin collagen state by multiphoton microscopy in the course of irradiation

N.D. Gladkova[1], V.V. Dudenkova[1,2], V.V. Elagin[1], K.V. Babak[3], A.V. Maslennikova[3,4]

[1]*State Medical Academy, Research institute of biomedical technologies, Nizhny Novgorod, Russian Federation*

[2]*Lobachevsky State University, General physics, Nizhny Novgorod, Russian Federation*

[3]*Lobachevsky State University, Biophysics, Nizhny Novgorod, Russian Federation*

[4]*State Medical Academy, Oncology and Radiotherapy beam diagnostics, Nizhny Novgorod, Russian Federation*

Purpose or Objective: Adverse events in normal tissues during and after a course of cancer radiation treatment are one of the most pressing problems of modern radiation oncology. From among numerous works in this field, there are but a few concerned with the radiation-induced alterations of collagen, the processes of its degradation and subsequent remodeling. A new imaging technique – multiphoton microscopy (MPM) allows studying tissue collagen state on fibers and bundles level without additional staining due to second harmonic generation (SHG) phenomenon. The method has the key advantage of a potential in vivo application. This study's objective was in vivo evaluation of changes occurring at rat's skin collagen upon the exposure of conventional irradiation.

Material and Methods: Rat's ear was chosen as a model for detecting collagen changes. Experiments were carried out under Nizhny Novgorod Medical Academy ethical committee permission. Three male animals, 2 months old at the time of experiment, were used. Rat's ear was irradiated under general anesthesia (Zoletyl, 50 mg/kg, Virbac Sante Animale, France) by a Co60 unit Terabalt (UJP, Czech Republic) by a local field with single dose of 2 Gy up to the total dose of 24 Gy. The 3D imaging of collagen structure was performed by MPTflex (JenaLab, Germany) – a system for in vivo optical biopsies based on near infrared femtosecond laser technology. MPM imaging was carried out two times a week beginning from the first day of irradiation and once a week for three months after its completion. Cross-sectional images were obtained beginning from the horny layer with the step of 5 μm up to the total depth of 100 μm. Excitation was implemented with a pulsed (200 fs) titanium-sapphire laser at a wavelength of 740 nm and a pulse repetition frequency of 80 MHz; SHG collagen imaging was performed at 373-387 nm. Cross-sectional images of 512x512 pixels were obtained; the field size was 130x130 μm. Numerical processing of the images was performed by ImageJ program. Mean fluorescence intensity and its standard deviation was calculated for all images. Coefficient S (a ratio of standard deviation/mean fluorescence intensity) was used for evaluation of collagen state.

Results:

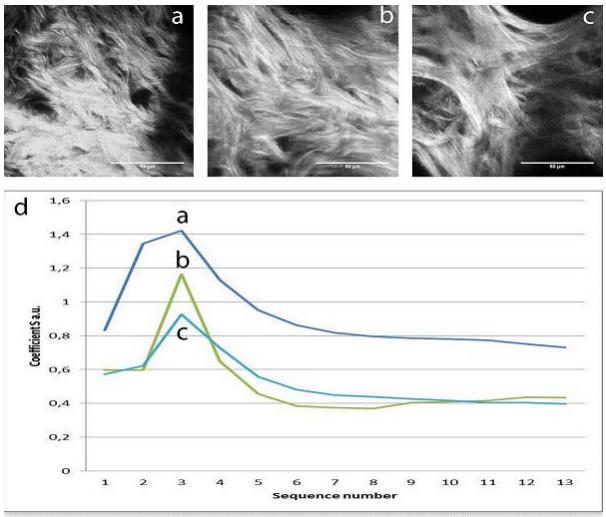

Fig.1. Examples of the MPM images of rat's ear: a - before irradiation, b - 24 Gy, c - 24 Gy, 3 month after irradiation, d - correspondent coefficient S.

Visual evaluation of MPM images demonstrated no noticeable changes of collagen packing and structure independent on the dose and time from radiation beginning (Fig.1, a, b, c). Numerical processing revealed subtle, but clear differences of coefficient S between intact and irradiated collagen. After radiation beginning, a decrease of magnitude of coefficient S and the decrease of title angle of the graph was observed (Fig.1 d). In a month after radiation completion, a magnitude remained decreased, but tilt angle of the graph returned to the initial level (Fig.1 d).

Conclusion: Numerical processing of MPM-images demonstrated changes of optical properties of collagen upon expose of clinically relevant doses of gamma-irradiation. The radiobiological interpretation of these changes require further study.

EP-2046

Modulation of radiation-induced oral mucositis (mouse) by dermatan sulfate

S. Gruber[1], E. Bozsaky[1], K. Frings[1], M. Arnold[1], V. Gernedl[1], S. Hetzendorfer[1], J. Mayer[1], S. Morava[1], S. Pfaffinger[1], P. Kuess[2], W. Dörr[1]

[1]*Medical University of Vienna, Department of Radiotherapy-ATRAB - Applied and Translational Radiobiology and Christian Doppler Laboratory for Medical Radiation Research for Radiation Oncology, Vienna, Austria*

[2]*Medical University of Vienna, Department of Radiation Oncology and Christian Doppler Laboratory for Medical Radiation Research for Radiation Oncology, Vienna, Austria*

Purpose or Objective: Oral mucositis is the most frequently occurring, dose limiting early adverse event of head-and-neck cancer radio(chemo)therapy. The purpose of the present study was to quantify the mucoprotective effect of dermatan sulfate (DS), and to characterise the associated changes in the expression of markers for epithelial proliferation, cell junctions, inflammation and hypoxia.

Material and Methods: The study comprises a functional and a histological arm. For the functional investigations, mice were irradiated with 5x3 Gy/week over one (days 0-4) or two weeks (days 0-4, 7-11). Each protocol was concluded by irradiation with graded top-up doses (day 7/14), to generate complete dose-effect curves. Daily doses of DS (4 mg/kg subcutaneously) were applied over varying time intervals. Mucosal ulceration, was analysed as clinically relevant endpoint during the functional studies. In the histological study, groups of three mice were sacrificed every second day, the tongues were excised and subjected to histological/immunohistochemical processing.

Results: DS significantly increased isoeffective doses for the induction of oral mucositis in almost all protocols, and

furthermore prolonged the latency to epithelial ulceration and reduced ulcer duration. Proliferation measurements with BrdU did not show any substantial effects of DS. The adherens junction protein ß-catenin did significantly increase during irradiation, which occurred earlier with additional DS treatment. The hypoxia markers HIF-1α and GLUT-1 showed a progressive increase during irradiaton alone, which, however, was also not influenced by DS. IL-1ß and NF-κB as markers of inflammation were dramatically increased during irradiation. While DS treatment abolished the radiation-induced increase of IL-1ß, however, no systematical effect on the expression of NF-κB was observed.

Conclusion: DS has a significant mucoprotective effect. This is not based on stimulation of epithelial proliferation nor on modulation of radiation-induced hypoxic changes. In contrast, reduced or modulated inflammatory processes and/or increased/modified function of adherens junctions may have a mechanistic role. This hypothesis, however, needs to be validated in further studies.

Electronic Poster: Radiobiology track: Biomarkers and biological imaging

EP-2047
1H NMR based metabolomic approach to monitoring of the head and neck cancer treatment toxicity

L. Boguszewicz[1], A. Hajduk[2], J. Mrochem-Kwarciak[3], A. Skorupa[1], M. Ciszek[1], A. Heyda[2], M. Sokol[1], K. Skladowski[2]
[1]*Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Department of Medical Physics, Gliwice, Poland*
[2]*Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, I Radiotherapy Clinic, Gliwice, Poland*
[3]*Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Analytics and Clinical Biochemistry Department, Gliwice, Poland*

Purpose or Objective: Anticancer treatment affects composition and concentrations of metabolites in body fluids. In case of head and neck (HNC) cancers the acute radiation syndrome (ARS) was studied only at the genomic, proteomic and lipidomic levels. We aimed to identify and investigate molecular processes of treatment toxicity in HNC patients using high resolution NMR and NMR-based metabolomics.

Material and Methods: Forty five patients with HNC were treated with radiotherapy (RT) or cisplatin-based chemoradiotherapy (CHRT). Blood samples were collected within a week after RT/CHRT completion. The ARS was evaluated using Multi-parameter Monitoring (MPM) – an original evaluation system designed by the study investigators. The patients were divided into two classes (of high and low ARS) on the basis of the highest individual ARS value observed during the treatment. The NMR spectra of the serum samples were acquired on 400.13 MHz Bruker spectrometer at 310 K. The referenced to alanine and bucketed to 0.002 ppm spectra were analyzed using principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). Additional statistical analyses (Mann-Whittney test, Pearson correlation) were performed on quantified metabolites.

Results: In the high ARS group we observed the increased signals of N-acetyl-glycoprotein – the NMR marker of inflammation, and acetate – a product of beta-oxidation of adipose tissue fatty acids. The high ARS group showed also the decreased signals of metabolites involved in energy metabolism: branched chain amino acids (BCAAs), alanine, creatinine, carnitine and glucose as well as decreased choline containing compounds reflecting disturbed membrane metabolism. Furthermore, we observed the positive correlations between C-reactive protein (CRP) and N-acetyl-glycoprotein as well as acetate and a percentage weight loss during the treatment. CRP was also negatively correlated with alanine and BCAAs.

Conclusion: 1H NMR is an efficient tool for detection of RT/CHRT toxicity markers in human serum. The results indicate at least three concomitant processes related to high treatment toxicity (high ARS): inflammation, altered energy metabolism and disturbed membrane metabolism. The combination of clinical and molecular approaches could deliver comprehensive information on treatment response, allowing monitoring and/or prediction of tolerance/toxicity of therapy as well as its outcome. Such approach gives a step forward into personalized therapy.

EP-2048
Serum cytokines as a predictive factor in hepatoma patients treated with radiotherapy

J. Seong[1], H. Cha[1], E.J. Lee[1]
[1]*Yonsei Cancer Center- Yonsei University Health System, radiation oncology, Seoul, Korea Republic of*

Purpose or Objective: Cytokines, which are involved in chronic inflammation, are also related to tumor aggressiveness and resistance to treatment in many cancers. However, there are limited reports on the significance of cytokines in tumor response to radiotherapy (RT). The aim of this study was to analyze serum cytokine levels and identify their association with treatment outcome in patients with hepatocellular carcinoma (HCC) treated with RT.

Material and Methods: Patients with HCC who treated with RT were eligible for this prospective study. Blood samples were collected before and after completion of the whole RT course. Serum cytokine levels measured using Cytokine Bead Array kits were analyzed with respect to patients' clinical profiles and treatment responses.

Results: Between September 2008 and October 2009, 51 patients were included in the analysis. Median follow-up duration was 12.3 months (range, 0.5-62.3). Forty-seven patients were diagnosed with modified UICC stage III or IV disease at the time of RT. Baseline serum IL-8 level increased with increasing stage and the IL-6 level was highest in patients with a history of pre-RT treatment (treatment-non-naïve). A higher baseline serum IL-6 level was also observed in patients with treatment failure, including overall, infield, and outfield failure, than in those without treatment failure. In subgroup analysis, a significant difference in serum IL-6 level was observed only in treatment-non-naïve versus treatment-naïve patients. Median overall survival and progression-free survival (PFS) were 13.9 and 7.7 months, respectively. Elevated serum IL-6 level was significantly associated with PFS for patients with infield failure (HR 1.011, $p<0.0001$).

Conclusion: The current findings suggest that assessment of baseline serum IL-6 level may be helpful to predict treatment outcome after RT for HCC, especially in patients who undergo treatment before RT.

EP-2049
Diffusion MRI for following tumor modifications after neoadjuvant radiotherapy

F. Lallemand[1], N. Leroi[2], M. Bahri[3], E. Balteau[3], A. Noel[4], P. Coucke[5], P. Martinive[5], A. Plenevaux[3]
[1]*University of Liège and CHU, Radiotherapy and Cyclotron Research Centre and Laboratory of Tumor and Development, Liège, Belgium*
[2]*University of Liège and CHU, Radiotherapy Laboratory of Tumor and Development, Liège, Belgium*
[3]*University of Liège, Cyclotron Research Centre, Liège, Belgium*
[4]*University of Liège, Laboratory of Tumor and Development Biology, Liège, Belgium*
[5]*University of Liège and CHU, Radiotherapy, Liège, Belgium*

Purpose or Objective: Neoadjuvant radiotherapy (NeoRT) improves tumor local control and tumor resection in many cancers. The timing between the end of the NeoRT and surgery is driven by the occurrence of side effects or the tumor downsizing. Some studies demonstrated that the